Molecular Therapy
Methods & Clinical Development
Commentary

A positive take on negative selection for CAR-T manufacturing

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https://doi.org/10.1016/j.omtm.2024.101218

Adoptive immunotherapy with engineered T cells has progressed at a lightning pace, resulting in approved indications for B cell malignancies, such as leukemia, lymphoma, and multiple myeloma, in both pediatric and adult populations. Due to this rapid pace, many fundamental questions have yet to be adequately addressed, including a seemingly obvious one: which cells should we use? While the processes for commercial chimeric antigen receptor (CAR)-T manufacturing remain a trade secret, a team of investigators at the National Institutes of Health, led by Dr. Steven Highfill, have begun to pull back the curtain. Song et al. demonstrate that how T cells are selected for genetic engineering leads to differences in the collection of phenotypes broadly grouped as "T cells" as well as the activation state of these cells.[1] This has implications for success in manufacturing CAR-T cells, CAR-T cell activity against disease, and toxicity associated with the therapy.

CAR-T cell manufacturing begins with the collection of a large number of peripheral blood mononuclear cells (PBMCs), usually by means of leukapheresis. Following leukapheresis, one may proceed directly to centrifugation over density gradient media, resulting in a fractionated PBMC population that has the majority of platelets, granulocytes, and serum removed. The mononuclear cells that remain are comprised of monocytes, natural killer cells, B lymphocytes, and T lymphocytes. At this stage, CAR-T manufacturing can begin. However, some of these cell types have been demonstrated to be detrimental. Work by this group at the NIH as well as others have clearly demonstrated that the presence of myeloid cells (expressing the CD14 surface marker) can inhibit the expansion of CAR-T cells during manufacturing.[2,3] Researchers at the University of Pennsylvania demonstrated that, on occasion, B cells can be transduced with the CAR-encoding gene vector—resulting in a leukemia cell that has the target antigen (CD19) down-modulated by virtue of co-expressing a CD19-specific CAR, which can render the leukemic cell population resistant to the very CAR designed to treat them.[4] Thus, the need to remove these cell types is paramount

Further purification of the starting cell population for CAR manufacturing can be approached by either positive or negative selection. Positive selection uses antibody-coated beads with an iron core that can be used to select cells by passing the treated cell population through a column or other matrix in the presence of a magnetic field. Smaller biodegradable matrices embedded with iron particles and antibody can also be used and have the advantage of not having to be removed from the cell population. Negative selection refers to selecting away unwanted cell populations and leaving the desired population untouched. The positive aspect of positive selection is having the ability to specify which markers will be used for selection. Positive selection for CAR-T manufacturing most often employs a selection matrix containing anti-CD4 and anti-CD8 antibodies, thus selecting only T cells expressing these markers. It would seem that this added specificity would be exactly what is required. However, there are two caveats. The first is that anytime a surface receptor is bound, it has the potential to signal the cell being selected. This is why simple CD3-based antibody binding for T cells has never been used to positively select a desired T cell population. In the report by Song et al., it was clearly demonstrated that using CD4 and CD8 binding approaches for T cell selection does impact T cell biology. This and previous reports have shown that engaging CD4 during selection increases the basal level of Erk phosphorylation and is associated with a 3-fold higher expression of CXCR4 on the positively selected cells and may account for higher levels of toxicity associated with positively selected cells administered, especially given the higher levels of interferon γ and interleukin-8 production by the positively selected population, as Song et al. demonstrate.[5,6]

The positive side to negative selection is further highlighted in this report. Cell types to which we may not yet have assigned anti-tumor activity are left in the cell population, most notably γδ T cells. γδ T cell activity in malignancy, recently reviewed by Wang et al., is an area of active investigation, and the presence and activity of γδ T cells is generally viewed as positive.[7] Due to their lack of an αβ T cell receptor, γδ T cells are often tested as additions to allogenic immunotherapy protocols. They also have been tested as cellular substrates for CAR-T expression and mediators for antibody-guided redirected killing (Table 1). While Song et al. do not definitively prove the association of γδ T cells with anti-leukemic effect, either as an "untouched" cellular population that comes along for the ride or as a positive anti-tumor effect mediated by CAR expression, they do show that some of the γδ T cells express the CAR and that they serve as effective killers of target cells *in vitro*.

The investigative group at the NIH that carried out this work has an established track record of iterative improvement in CAR-T manufacturing and therapeutic analysis of its impact. Here, they have given us important new ways to analyze donor variability, have demonstrated the normalization of donor activity according to the cell

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Table 1. Inclusion γδ T cells enhances anti-tumor immunity

γδ T cell therapy	Indication	Reference/clinical trial
Neuroblastoma	γδ T cells added to anti-GD2 (dinutuximab) therapy	NCT05400603, Jonus et al.[9]
Allo-HSCT	infusion of γδ T cells following post-transplant cyclophosphamide	NCT03533816, McGuirk et al., 2023, ASH no. 4853
AML post-allo-HSCT	presence of bone marrow γδ T cells associated with complete response	Mathioudaki et al.[10]
Spatial biology investigation	colorectal carcinoma tissue study	Herold N. et al.[11]
CD7 γδ CAR-T	T cell leukemia/lymphoma	NCT04702841
NKG2DL γδ CAR-T	solid tumors and heme-malignancies	NCT05302037
CD19 γδ CAR-T	B-ALL and CLL	NCT02656147

manufacturing protocol, have again highlighted that the "process is the product" in cell manufacturing, and have opened the door to further cell manufacturing innovation. The group was limited by using a pre-determined antibody mix for negative depletion from a commercial supplier. While changing the antibodies used in this approach is difficult due to the requirement for good manufacturing practice sourcing, others are also beginning to evaluate how cell negative depletion approaches can be both optimized and simplified.[8] Given the chance to further define other cell types that remain using a negative selection approach opens the door to continued investigation of the contribution that different mononuclear cell types make to anti-cancer activity in engineered cellular populations.

DECLARATION OF INTERESTS

R.J.O. serves on the scientific advisory board of Umoja Biopharma and Galapagos NV.

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